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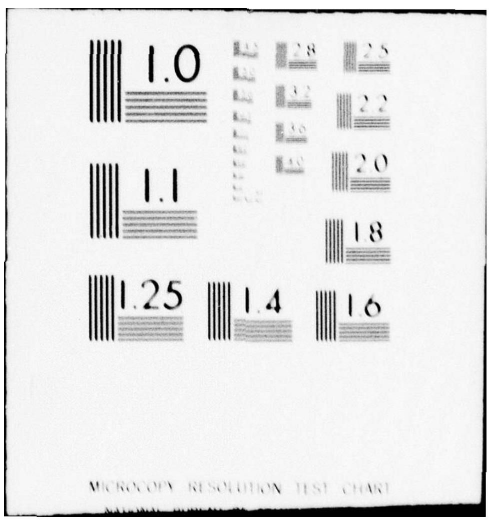
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The Pennsylvania State University

The Graduate School

Department of Comparative Medicine

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The Effect of Chronic Parenteral Carbohydrate Administration on Drug Metabolism in the Rat,

9 Master's ~~A~~ Thesis, in

Laboratory Animal Medicine

by

10 Rodney Daryl Hartshorn

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

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April 18, 1978	William J. White, Assistant Professor of Comparative Medicine, Graduate Faculty Reader

The Effect of Chronic Parenteral Carbohydrate Administration on Drug Metabolism in the Rat 79-69T

Rodney Darrell Hartshorn - Master of Science May 78

Pennsylvania State University.

ABSTRACT

Dietary factors can affect the liver's ability to metabolize drugs; however, few studies have been done to show the effects of parenteral nutrition on drug metabolism. The effects of chronic parenteral carbohydrate administration on hepatic microsomal enzyme activity were studied in the rat. Daily intraperitoneal injections of both glucose and fructose resulted in a significantly decreased cytochrome P-450 content with subsequent losses in mixed function oxidase activity (ethylmorphine N-demethylation) by the fifth day. Aniline hydroxylation, however, was not significantly decreased until the seventh day, suggesting that the hepatic metabolism of ethylmorphine and aniline is mediated through different forms of cytochrome P-450. *In vivo* assessment using antipyrine half-lives confirmed this decrease in mixed function oxidase activity.

The administration of carbohydrate produced fatty infiltration and glycogen depletion of the liver. This fatty infiltration was probably due to increased lipogenesis with decreased oxidative metabolism of fat by the liver, since all groups received the identical quantity of specific nutrients. The glycogen depletion may have been due to increased hepatic cyclic AMP.

The decreased hepatic mixed function oxidase activity could have been the result of sustained hyperglycemia, hypoinsulinemia, and/or increased levels of hepatic cyclic AMP.

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LIST OF ABBREVIATIONS

a.m.	ante meridiem
AMP	adenosine monophosphate
C	centrigrade
cm	centimeter
-COOH	carboxyl group
CPM	counts per minute
dl	decaliter
g	a unit of force applied to a body at rest equal to the force exerted on it by gravity
gm	gram
H & E	hematoxylin-eosin
H_2O	water
M	molar
MCR	metabolic clearance rate
MFO	mixed function oxidase
μCi	microcurie
μmol	micromolar
mg	milligram
ml	milliliter
mm	millimeter
mM	millimolar
min	minute
N	normal
$NADP^+$	nicotinamide adenine dinucleotide phosphate, oxidized form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
$-NH_2$	amino group
nmol	nanomole
p	probability
PAS	periodic acid-Schiff
pH	the negative logarithm of the effective hydrogen ion concentration
rpm	revolutions per minute
-SH	sulfhydryl group
t 1/2	plasma half-life
t_o	theoretical plasma concentration at zero time
Tris-HCl	Tris (hydroxymethyl) aminoemthane-hydrochloride
V_d	volume of distribution
%	percent

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I am especially indebted to my wife for her love, support, and patience during this period of my life.

INTRODUCTION

Many drugs and other foreign compounds are biotransformed by microsomal enzymes located predominantly within the endoplasmic reticulum of the liver. This biotransformation usually involves the chemical alteration of a lipid soluble compound into a less lipid soluble, and hence more polar, compound which can be eliminated by the kidney. The rate at which hepatic microsomal enzymes metabolize an exogenous compound affects the duration and intensity of that compound's action in the living system (14). A single compound may undergo several biotransformations in a sequential fashion. These reactions are oxidations, reductions, hydrolyses, and conjugations. The specific chemical reactions in which compounds are altered are many and varied, but they can be divided into two main categories: synthetic reaction and nonsynthetic reactions (49).

Synthetic reactions, also known as conjugations, result in alteration of the parent compound by combining them with endogenous molecules provided by the body. These molecules, known as conjugating agents, are usually carbohydrates, amino acids, or substances derived from these nutrients. Whether or not a particular compound will readily combine with a conjugating agent is determined by the structure of that parent compound. If this foreign chemical possesses an appropriate center for conjugation, such as a carboxyl (-COOH), hydroxyl (-OH), amino ($-NH_2$) or sulfhydryl (-SH) group, a synthetic reaction is likely to occur. If the parent compound does not possess such a functional group, it may first acquire one by a non-synthetic reaction (49). Synthetic reactions usually result in termination of the biological activity of drugs and their metabolites

(45). The compounds resulting from synthetic reactions are readily eliminated in urine and feces, since conjugated molecules are usually highly ionized acids and are relatively lipid insoluble. Synthetic reactions occurring in man are acetylations, methylations, and conjugations to sulfate, amino acids and glucuronic acid.

The nonsynthetic reactions involve chemical alteration of the parent compound by oxidation, reduction, hydrolysis, or a combination of these processes. Unlike synthetic reactions, nonsynthetic reactions do not necessarily result in pharmacologically inactive products. The nonsynthetic processes may actually convert an inactive drug to an active compound, or change an active drug into another pharmacologically active compound (37). End products of nonsynthetic reactions are generally not excreted from the body, but usually become substrate for synthetic reactions. Thus, most drugs undergo a two-stage biotransformation; the first phase is the addition of an appropriate center for conjugation to the parent compound, and the second is the combination of this altered parent compound with a suitable conjugating agent.

The majority of the nonsynthetic reactions occur within the smooth endoplasmic reticulum of the hepatocyte. These are catalyzed by an enzyme complex which, when experimentally fractionated and prepared as "microsomes," is conventionally classified as a mixed function oxidase (MFO) system (51). This enzyme complex includes a hemeprotein (cytochrome P-450) and a flavoprotein (nicotinamide adenine dinucleotide phosphate [NADPH]-cytochrome c reductase), that require both oxygen and a reducing agent (NADPH) to function. These microsomes possess a broad substrate "specificity," since a wide variety of drugs, exogenous compounds and endogenous compounds, such as steroid hormones, may serve as metabolic substrates for these enzymes (49,61).

Many environmental factors, including bedding, cleanliness of housing, hormonal changes and the ingestion of foreign compounds, can either stimulate or inhibit the liver's capacity to metabolize exogenous compounds (14,47). Recent findings have shown that the composition of the diet can also be an important environmental determinant of the pharmacological and toxicological properties of drugs and toxins (11,12). The influence of the individual macronutrients (carbohydrates, lipids, and proteins) on hepatic drug metabolism, however, is difficult to assess for a variety of reasons (12), including variabilities in the caloric intake and composition of the diet, possible stress effects created by over- or under-supplementation of nutrients, and "sparing" actions that result from the supplementation of one macronutrient upon the deficiency of another. Thus, although many studies have shown a dietary effect on drug metabolism, little is known about the biochemical and physiological mechanisms involved.

The dietary constituent most frequently studied in relation to hepatic microsomal enzyme activities is protein. A reduction in the quantity or quality of dietary protein has been shown to decrease hepatic microsomal enzyme activity (31,32,53,54). This results in decreased microsomal oxidations of many drugs and other foreign compounds, such as pesticides, with a subsequent increase in their toxicity (3,7,38,40,42,43). The decreases in microsomal oxidations are due, at least in part, to decreases in cytochrome P-450 (11). In contrast, a high protein diet increases the rate at which drugs are metabolized in animals (40,43).

Lipid substances in the diet may be as important as protein in affecting microsomal activity. Studies by Norred and Wade (57) have shown that changes in the lipid composition of the microsomal membranes alter the ability of the microsomal enzymes to bind substrate. Other studies have shown that endogenous lipids, such as steroids (44,68) and fatty acids (15,16) may occupy cytochrome P-450 binding sites, thereby displacing exogenous substrates and perhaps interfering with their metabolism.

A specific biochemical role for carbohydrates in the MFO system has not been demonstrated; however, changes in dietary carbohydrate composition have been shown to produce alterations in this enzyme system (11,12). Studies have shown that dietary manipulation of carbohydrates appear to affect the microsomes through generalized actions on intermediary metabolism, caloric intake, and/or hormonal-stress relationships (11). Boyd *et al*. (8) found that high sucrose diets potentiated the toxic effect of benzylpenicillin. The investigators suggested that this was the result of a lower rate of conversion of the drug to a less toxic product. Jansson *et al*. (36) reported that a high carbohydrate intake inhibits lipid peroxidase activity and decreases hepatic MFO activity in rats. This decrease in microsomal MFO activities, and a subsequent increase in the pharmacological action of administered drugs, has recently been demonstrated in humans fed a high carbohydrate diet (3,38).

Strother *et al*. (72) have shown that a high dietary intake of various sugars (glucose, sucrose or fructose) will increase the duration of sleep induced by barbiturates in mice. This effect was

most pronounced with glucose. The longer sleeping times were correlated with a decrease in the rate of metabolism of the barbiturates. This effect of high glucose was transitory, lasting only three to four days, after which time the mice returned to their original sleeping times. The animals consuming a high glucose diet did not have a significant elevation in blood glucose; however, the investigators did not specify when the animals had eaten. They further stated that urinary glucose greater than 2% occurred overnight. Therefore, although hyperglycemia did not appear to be related to the observed effect, it is possible that hyperglycemia may have been a factor at some point in their study. In an attempt to establish the mechanism for the increased sleeping time, Peters _et al._ (63) observed that high dietary glucose levels significantly decreased NADPH oxidase activity and nonsignificantly, but reproducibly, decreased cytochrome P-450 content, cytochrome P-450 reductase activity and NADPH-cytochrome c reductase activity. They concluded that decreased hepatic microsomal barbiturate metabolism was responsible for the prolongated barbiturate sleeping time after glucose treatment.

Two studies have shown that high carbohydrate diets decrease hepatic MFO activity in rats by altering cytochrome P-450 levels. Dickerson _et al._ (18) found that the addition of fructose to a high glucose diet significantly lowered cytochrome P-450 content. Later, Basu _et al._ (5) reported that the dietary substitution of starch by sucrose or its constituent monosaccharides, glucose and fructose, significantly decreased aromatic hydroxylase activity and cytochrome P-450 content in the hepatic microsomes of young rats.

To date, all studies in experimental animals (5,8,18,36,63,72) and man (3,38), where a high carbohydrate diet has been found to decrease hepatic MFO activity, contain one major deficiency in experimental design. None of the experiments ensured that both experimental and control groups received the same quantity of protein, even though many of the studies attempted to control the total calories consumed. This problem in experimental design is particularly important when the work of both Peters (63) and Strother (72) is taken into consideration. Both of these investigators found that when a 30% glucose solution was given in place of drinking water, a 40% reduction in food intake occurred. Therefore, under the conditions of their studies, decreased hepatic drug metabolism could have arisen not primarily from increased dietary carbohydrate, but rather secondarily from subsequently decreased protein consumption.

From the above it is obvious that dietary factors can affect the liver's ability to metabolize drugs, even though the mechanisms involved seem obscure at the present time. In human and veterinary medicine parenteral nutrition is used routinely in patients. This nutrition can vary from a sterile water solution containing 5% dextrose to a hyperosmolar solution containing amino acids and fat emulsions (25). Very few studies have been done to show the effects of parenteral nutrition on drug metabolism. Several investigators have reported that parenteral carbohydrates produce transient pharmacokinetic effects, i.e. effects on drug distribution, biotransformation, and/or excretion (46,50). Lamson _et al_. (46) showed that, shortly after waking, approximately 50% of dogs anesthesized with pentobarbital returned to sleep

after intravenous glucose. The mechanisms for this "glucose effect" have yet to be determined. Other investigators have shown that fructose administered intravenously accelerates alcohol metabolism in man (10,50). Since parenteral nutrition is usually accompanied by parenteral or per os drug administration, the opportunity for interaction occurs frequently. Therefore, it becomes important to know what pharmacokinetic effects, if any, are produced by these nutrient solutions. The purpose of this study was to determine if chronic parenteral carbohydrate administration alters hepatic microsomal enzyme activity and drug metabolism in the rat.

MATERIALS AND METHODS

Animal Studies

All animals used for this study were adult male rats[1] weighing 180 to 200 grams each. The rats were quarantined for one week prior to study and were fed a commercial laboratory chow[2] and water *ad libitum* prior to experimentation. Throughout the investigation the rats were housed in groups of six in stainless steel wire cages suspended over absorbent paper bedding.[3] The absorbent paper bedding and trays were changed daily to avoid the influence of exposure to waste products on liver microsomal enzymes (75). The temperature in the cubicle was maintained at 72 ± 2°F with a 50 ± 10% relative humidity and 23 ± 1 air changes per hour using non-recycled air. The photoperiod was maintained at 12-hours light and 12-hours dark with no twilight. In addition, all rats were handled identically throughout the entire experiment to eliminate physical stress as a variable between groups (73).

The hepatic microsome study was divided into two phases (Table 1). The first phase had three treatment groups of six rats each. The treatments were either glucose, fructose, or saline injections given intraperitoneally at three-hour intervals over the entire time periods studied. The treatment groups received their injections for 2, 5, or

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[1] Crl:COBS® CD® (SD), Charles River Breeding Laboratories, Inc., Wilmington, MA 01887.

[2] Purina Laboratory Chow®, Ralston Purina Company, Checkerboard Square, St. Louis, MO 63188.

[3] Cageboard®, The Upjohn Company, Kalamazoo, MI 49001.

TABLE 1. Designation of Groups According to Treatment

Study	Treatment Group	Number of Animals per Group 2 days of Treatment	5 days of Treatment	7 days of Treatment
Hepatic Microsome				
Phase I	glucose	6	6	6
	fructose	6	6	6
	saline	6	6	6
Phase II	xylose	-	-	6
	saline	-	-	6
Liver Glycogen and Histology	glucose	-	-	6
	fructose	-	-	6
	saline	-	-	6
Antipyrine Half-Life	glucose	-	-	6
	fructose	-	-	6
	saline	-	-	6
Serum Hexose	glucose	12	-	-
	fructose	12	-	-
	saline	4	-	-

7 days. Phase II consisted of two groups of six rats each, receiving either saline or xylose intraperitoneally at three-hour intervals for seven days. The liver glycogen study consisted of three groups of six rats each, receiving either glucose, fructose, or saline injections intraperitoneally for seven days. On the morning following the last treatment, the animals in the above groups were euthanized by decapitation. The livers from rats in the hepatic microsome study were collected for hepatic microsomal assays, while the livers from the liver glycogen study were collected for liver glycogen assays and histologic examination.

The antipyrine half-life study (Table 1) consisted of three treatment groups of six rats each. Each group received either glucose, fructose, or saline at three-hour intervals for seven days. On day 8 of the study, plasma antipyrine half-lives were determined.

A blood hexose study was done to determine if the serum glucose, fructose, and protein levels were affected by repeated treatments with glucose or fructose. This study was divided into three groups (Table 1). Groups 1 and 2 consisted of 12 rats each, that received either glucose or fructose intraperitoneally at three-hour intervals for 48 hours, and group 3 consisted of four rats that received saline intraperitoneally at three-hour intervals for two days. The glucose- and fructose-treated rats were killed by decapitation at 1-, 2-, and 3-hour intervals following post-injection; the saline-treated rats were killed one-hour post-injection. Blood was collected in 15-ml centrifuge tubes and centrifuged at 3000 rpm for ten minutes. The serum was then removed with Pasteur pipettes and stored in 8-ml polyethylene specimen vials at $-70^{\circ}C$ until assayed.

The dosage schedule for all experimentally-treated animals was 1 ml of a 1-M solution per 100 grams body weight. This dosage was based on preliminary information gathered by this author using rats injected with a 1-M glucose solution. One milliliter of a 1-M glucose solution was shown to maintain a constant hyperglycemia in the range of 150 to 185 mg/dl. Control groups received 1 ml of 0.9% saline per 100 grams body weight of 0.9% saline.

All rats received the proper quantity of nutrients necessary to maintain normal body growth and function (2). Their caloric and nutrient intake was controlled so that all groups received an identical number of calories, as well as amount of protein and carbohydrate. Each rat was fed 6 ml of a liquid diet (Table 2) twice daily with an animal intubation needle (3 in, 16 ga, curved, with a 3-mm ball).[4] The liquid diet was prepared fresh daily by adding 1 gram of diet powder to 1.3 ml of water. This mixture provided 2.1 calories per ml of diet (69). Since the rats in the glucose and fructose groups received an additional 11.52 calories of carbohydrate per day intraperitoneally, the diet for the saline-control groups was made isocaloric by supplementation with an additional 2.88 grams dextrin, to provide the extra 11.52 calories. Because xylose is not metabolized in the rat (17), the saline-control group's diet in phase II of the hepatic microsome study was not supplemented with dextrin. All rats were weighed at the start of the study and immediately prior to euthanasia.

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[4]Popper and Sons, Inc., New Hyde Park, NY 11040.

TABLE 2. Composition of Tube Feeding Rat Diet, Modified® [1]

Vitamin Free Casein	20.0%
L-Cystine	0.3%
Dextrin, White	67.0%
Corn Oil	8.0%
Salt Mixture[a] + Vitamin Mixture[b]	4.0%

[a]Salt Mixture Composition

Calcium Carbonate	54.300%
Magnesium Carbonate	2.500%
Magnesium Sulfate·7 H_2O	1.600%
Sodium Chloride	6.900%
Potassium Chloride	11.200%
Potassium Phosphate (monobasic)	21.200%
Ferric Phosphate	2.050%
Manganese Sulfate·H_2O	0.035%
Sodium Fluoride	0.100%
Aluminum Potassium Sulfate	0.017%
Copper Sulfate·5 H_2O	0.090%
Potassium Iodide	0.008%

[b]Vitamin Mixture (gm/100 lb)

Vit A Conc. (200,000 units/gm)	4.500
Vit D Conc. (400,000 units/gm)	0.250
Alpha Tocopherol	5.000
Ascorbic Acid	45.000
Inositol	5.000
Choline Chloride	75.000
Menadione	2.250
p-Aminobenzoic Acid	5.000
Niacin	4.500
Riboflavin	1.000
Pyridoxine Hydrochloride	1.000
Thiamine Hydrochloride	1.000
Calcium Pantothenate	3.000
Biotin	0.020
Folic Acid	0.090
Vitamin B-12	0.135

[1]Tube Feeding Rat Diet, Modified®, ICN Pharmaceuticals, Inc., Cleveland, OH 44128

Preparation of Microsomes

Between 7 and 8 a.m. on the morning following each phase of the study, the rats were killed by decapitation after a 12-hour fast. The livers were removed, weighed, and placed in two volumes of ice cold 1.15% KCl solution buffered with 0.02 M Tris-HCl (pH 7.4). All subsequent tissue manipulations were carried out at 0 to $4^{o}C$. The livers were homogenized by six passes in a glass homogenizer having a motor-driven teflon pestle. After homogenization, the suspensions were transferred to polypropylene centrifuge tubes and spun at 9000 x g for 20 minutes in a refrigerated centrifuge. After aspiration of the fatty layer from the top, the supernatant fractions were carefully removed, avoiding the pellet. The supernatant was then centrifuged at 78,000 x g in a refrigerated ultracentrifuge for 60 minutes. The 78,000 supernatant fractions were again carefully removed, allowing the fluffy layers to remain undisturbed with the pellets. These remaining microsomal pellets were resuspended in the buffered 1.15% KCl solution using a glass homogenizer (74). The final protein concentration after resuspension was approximately 10 mg/ml as determined by the biuret method of Gornall *et al.* (27).

Analytical Methods

The cytochrome P-450 content was determined by the dithionite difference method of Omura and Sato (60), using a dual-beam spectrophotometer. An extinction coefficient of 91 $mM^{-1}cm^{-1}$ was used for the determination of the cytochrome P-450 content, which was done immediately after isolation of the microsomes.

Ethylmorphine N-demethylase activity was assayed in 3 ml of an incubation mixture consisting of 15 μmol ethylmorphine hydrochloride[5], 1.31 μmol of $NADP^{+}$[6], 25 μmol of magnesium chloride, 32.8 μmol of glucose-6-phosphate[6], and 0.99 units of glucose-6-phosphate dehydrogenase.[6] The concentration of protein was 5 mg per incubation vial. The measurement of formaldehyde produced during the demethylation process was done according to the method of Nash (56), with correction being made for the apparent formaldehyde formed in the absence of substrate (28). Incubations were performed in air at 37°C, using a shaking incubation (120 oscillations per minute) for 12 minutes.

Aniline hydroxylase activity was assayed in 3 ml of an incubation mixture consisting of 65 μmol of aniline hydrochloride[7], 1.31 μmol of $NADP^{+}$, 25 μmol of magnesium chloride, 32.8 μmol of glucose-6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase. The concentration of protein was 10 mg per incubation vial. Aniline hydroxylase activity was monitored by assay of the p-aminophenol formed as described by Chhabra *et al*. (13). The mixture was incubated in air at 37°C for 22 minutes (120 oscillations per minute).

Serum glucose concentrations were determined by an enzymatic method utilizing glucose oxidase to oxidize glucose to gluconic acid (41). This assay is specific for glucose and will not measure serum fructose (34). Serum fructose was determined by the Seliwanoff

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[5] Merck and Co., Rahway, NJ 07065.

[6] Sigma Chemical Co., Phillipsburg, NJ 08865.

[7] Eastman Organic Chemicals, Rochester, NY 14602.

reaction, modified by Roe (65,66), utilizing resorcinol as the reagent. Total serum proteins were assayed by the biuret method (27).

Liver glycogen was assayed after seven days of injections by the method of Montgomery (55), following digestion of fresh liver slices with 30% potassium hydroxide.

Histologic Procedures

Liver sections were obtained immediately after death and placed in 10% neutral buffered formalin solution. Five-micron sections from paraffin-embedded blocks were stained with either hematoxylin-eosin (H & E) or periodic acid-Schiff (PAS) for histologic examination (35). In addition, frozen sections were cut at five microns from non-embedded tissue and impregnated with 1% osmium tetroxide solution for fat differentiation. All sections were prepared and examined using a double-blind technique.

Plasma Antipyrine Half-Life Assay

The antipyrine half-lives were assayed using a purified solution of ^{14}C-labeled antipyrine[8] (2 μCi) and unlabeled antipyrine[9] (24 mg) in 1 ml of 0.9% saline. The purified solution was obtained by extracting 10-ml aliquots of the solution in 3-ml chloroform to which 5 ml of 1-N NaOH had been added. After manual shaking for 15 minutes, the chloroform phase was evaporated under nitrogen at 60°C and redissolved in 0.9% saline. Radiochemical purity greater than 98% was determined by thin layer chromatography and autoradiography. Silica gel G plates

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[8] Antipyrine-N-methyl-^{14}C, 10-20 μCi/mmol, New England Nuclear, Pilot Chemicals Division, Boston, MA 02118.

[9] Matheson Coleman and Bell Manufacturing Chemists, Norwood, OH 45212.

were used with a solvent system of 50 parts of butyl acetate, 40 parts of acetone, 30 parts of n-butanol, and 10 parts of ammonium hydroxide (70).

Between 7 and 9 a.m. on the eighth day of the study, the antipyrine half-life groups were given an intraperitoneal injection of the purified ^{14}C-antipyrine (2 μCi per 100 grams body weight). Blood samples were collected at 30, 60, 120, 180, and 240 minutes from the medial canthi of the rats' eyes. Each sample (0.1 ml) was then added to a solution containing 3 ml of chloroform and 1 ml of 1-N NaOH. After 15 minutes of manual shaking the solution was centrifuged at 500 x g for ten minutes. One milliliter of the chloroform phase was removed, placed in a scintillation vial with 10 ml of quencher[10] and counted in a liquid scintillation counter. Counting efficiency was 95-97%. It was not necessary to separate unchanged ^{14}C-antipyrine from any ^{14}C-containing metabolites, using thin layer chromatography. This was because a previous study (4) had shown that the 3-hydroxymethyl derivative is the only radioactive metabolite which is extracted together with antipyrine at alkaline pH using chloroform. Radiochromatogram scanning of the extracts showed that this compound caused very little over-estimation of ^{14}C-antipyrine.

A straight line was obtained when counts per minute (CPM) were plotted against time on semilog paper. The results, except for the pharmacokinetic analysis, were expressed as the half-life for elimination of antipyrine in serum.

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[10]Aquasol®, New England Nuclear, Pilot Chemicals Division, Boston, MA 02118.

Pharmacokinetic Analysis

The pharmacokinetic analysis (21,58) was based on the assumption that antipyrine fits the two-compartment model in rats, with α, or the distribution phase, being too fast to measure under experimental conditions. The half-life for elimination of the drug was calculated by least squares analysis. To determine the apparent volume of distribution (V_d), linear regression analysis was performed to calculate the extrapolated y intercept, which represented the theoretical plasma concentration at zero time (t_o):

$$V_d = \frac{\text{dose administered in mg}}{\text{plasma level at } t_o}$$

The metabolic clearance rate (MCR) was then determined by using the formula

$$MCR = \frac{0.693 \times V_d}{t\ 1/2}$$

where t 1/2 is the plasma half-life of antipyrine.

Methods of Data Analysis

The microsomal enzyme activities were first expressed as nanomoles of p-aminophenol formed per mg microsomal protein per minute for aniline hydroxylase and nanomoles of formaldehyde formed per mg of microsomal protein per minute for ethylmorphine. Cytochrome P-450 content was expressed as nanomoles of cytochrome P-450 per mg of microsomal protein. In order to compare the effects of glucose and fructose administration on these mixed function oxidase activities at the specified time intervals, the results were expressed as percent of control.

Fatty infiltration of the liver was evaluated using a double-blind technique. The degree of fatty change was rated on a scale of 1 to 4 (i.e. none, mild,, moderate, and severe).

All statistical analyses were performed using the Student's t test (26). Values of $p < 0.05$ were considered to represent differences between means.

RESULTS

Hepatic Microsome Study

Growth and Liver Weight

All rats remained healthy during the course of the study. None appeared to suffer complications from the repeated intraperitoneal injections and the twice-daily stomach intubations. Subjectively, the fructose and saline-control groups appeared to be more active, while the glucose groups were less active during arousal from sleep.

All rats gained weight in a linear fashion, averaging approximately three grams per day. Liver weights increased more rapidly in the glucose- and fructose-treated rats than in the saline controls (Table 3). The liver weights were significantly greater ($p < 0.05$) in carbohydrate-treated animals than in the saline-treated controls after five and seven days.

Phase I - Microsomal Assays

Microsomal data for MFO activities from the glucose-, fructose-, and saline-treated rats are shown in Table 4. The primary data was expressed as percent of concurrently-analyzed saline controls. This was done to account for diurnal variations, seasonal rhythms, housing conditions, etc. (47).

After two days of carbohydrate administration, all microsomal values, with the exception of aniline hydroxylase activity, were slightly lower than the saline-treated controls. By five days all values continued to decrease. Cytochrome P-450 content and ethylmorphine N-demethylase activity decreased significantly ($p < 0.05$) to

TABLE 3. Comparison of Liver Weights for Glucose-, Fructose-, and Saline-Treated Rats

Treatment	Days of Administration	Liver Weight Grams	Liver Weight Percent of Body Weight	Liver Weight Percent of Control[a]
Glucose	2	7.05 ± 0.18[b]	3.60 ± 0.07	103.45
	5	7.77 ± 0.17[c]	3.90 ± 0.07[c]	108.33[c]
	7	8.28 ± 0.22[c]	3.88 ± 0.19[c]	109.30[c]
Fructose	2	6.85 ± 0.16	3.55 ± 0.04	102.01
	5	7.92 ± 0.16[c]	3.99 ± 0.07[c]	110.83[c]
	7	8.20 ± 0.26[c]	4.02 ± 0.06	113.24[c]
Saline	2	6.78 ± 0.10	3.48 ± 0.03	-
	5	7.17 ± 0.18	3.60 ± 0.05	-
	7	7.00 ± 0.09	3.55 ± 0.19	-

[a] $\frac{\text{Liver weight}}{\text{Body weight}}$, expressed as percent of saline-treated controls.

[b] Mean ± Standard Error.

[c] Significantly different from corresponding saline-treated controls, $p < 0.05$.

TABLE 4. Comparison of Microsomal Values for Glucose-, Fructose-, and Saline-Treated Rats

	Days of Administration	Microsomal Protein Concentration[a]	Cytochrome P-450[b]	Ethylmorphine Demethylase[c]	Aniline Hydroxylase[c]
Glucose-Treated	2	24.86 ± 0.40[d]	0.412 ± 0.011	4.80 ± 0.29	0.350 ± 0.022
	5	23.34 ± 0.26	0.346 ± 0.012[e]	4.76 ± 0.27[e]	0.397 ± 0.024
	7	22.50 ± 0.29[e]	0.284 ± 0.011[e]	3.74 ± 0.32[e]	0.352 ± 0.018[e]
Fructose-Treated	2	25.28 ± 0.35	0.417 ± 0.012	4.89 ± 0.18	0.364 ± 0.026
	5	22.94 ± 0.27	0.374 ± 0.013[e]	4.98 ± 0.31[e]	0.405 ± 0.019
	7	22.28 ± 0.23[e]	0.281 ± 0.015[e]	3.90 ± 0.47[e]	0.364 ± 0.022[e]
Saline-Treated	2	25.58 ± 0.36	0.465 ± 0.020	5.75 ± 0.40	0.356 ± 0.035
	5	24.14 ± 0.44	0.493 ± 0.018	7.21 ± 0.51	0.462 ± 0.022
	7	24.20 ± 0.31	0.440 ± 0.022	6.53 ± 0.39	0.458 ± 0.020

[a]Expressed as mg protein per gram liver.

[b]Expressed as nmol of cytochrome P-450 per mg microsomal protein.

[c]Expressed as nmol product formed per mg microsomal protein per minute.

[d]Mean ± Standard Error.

[e]Significantly different from corresponding saline-treated controls, $p < 0.05$.

70% and 66% of control, respectively, for the glucose-treated group, and to 76% and 69% for the fructose-treated group (Figure 1). After seven days the cytochrome P-450 content and ethylmorphine N-demethylase activity further decreased to 65% and 57% for the glucose group and to 64% and 60% for the fructose group.

Aniline hydroxylase activity response was similar, but at a slower rate of decline, and was not significantly lower ($p < 0.05$) until day seven of the study. At this time, the activity was 77% and 79% of control values for the glucose and fructose groups, respectively.

The microsomal protein concentration of the liver slowly decreased during the study becoming significantly lower ($p < 0.05$) than the saline-treated controls by day seven, when the glucose-treated group was 93% and the fructose-treated group was 92% of control values.

During the entire seven-day study, the glucose and fructose groups displayed a similar response. The cytochrome P-450 content, ethylmorphine N-demethylase activity, aniline hydroxylase activity, and microsomal protein concentration from ´oth groups were not significantly different from each other during any particular day.

Phase II - Hyperosmolar Effect Study

The weight gains in both the xylose- and saline-treated rats were similar to that observed in Phase I (Table 5). In addition, there were no significant differences in liver weight between groups. The microsomal data for all groups were also similar, indicating that MFO activity was the same in both experimental and control rats (Table 6).

Liver Glycogen and Histology

Mild to moderate fatty changes (i.e. fatty infiltration) were noted in the livers of all the glucose- and fructose-treated rats;

Figure 1. Mean microsomal values for glucose-and fructose-treated rats expressed as percent of saline-treated controls

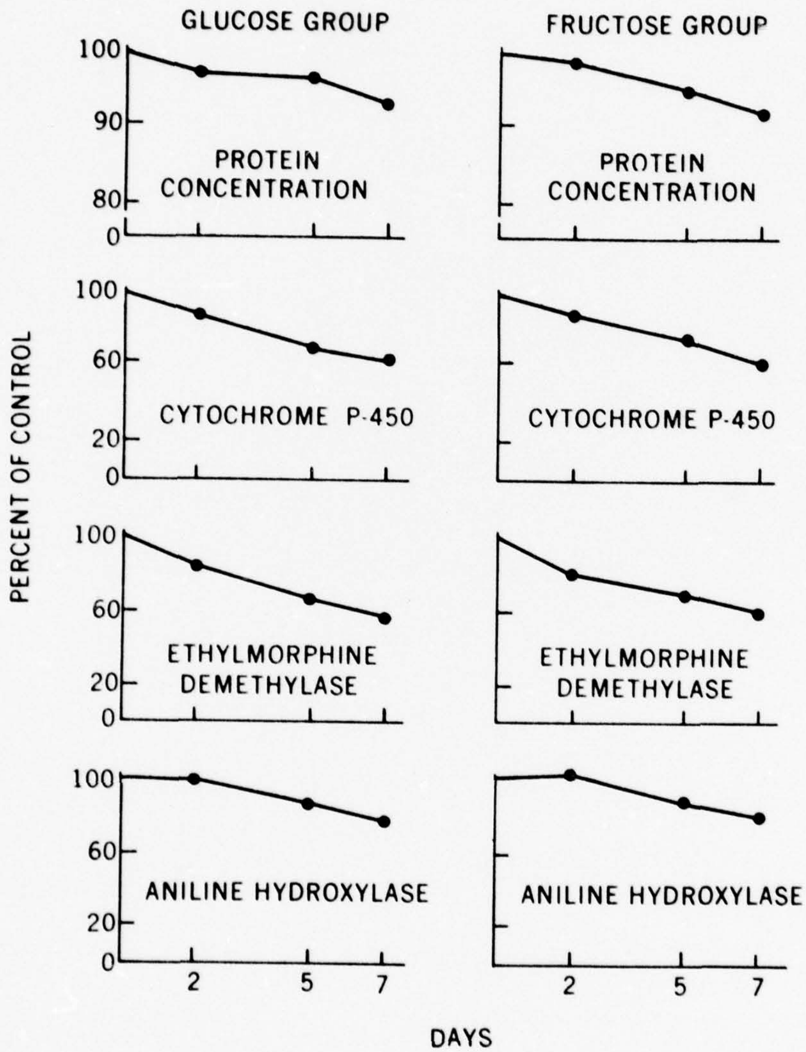
GLUCOSE GROUP
FRUCTOSE GROUP
PROTEIN CONCENTRATION
CYTOCHROME P-450
ETHYLMORPHINE DEMETHYLASE
ANILINE HYDROXYLASE
PERCENT OF CONTROL
100
90
80
60
20
0
2
5
7
DAYS

TABLE 5. Comparison of Weight Gain and Liver Weight for Xylose- and Saline-Treated Rats

	Days of Administration	Body Weight Increase (gm)	Liver Weight		
			Grams	Percent of Body Weight	Percent of Control[a]
Xylose-Treated	7	22.7 ± 1.3[b]	7.50 ± 0.19	3.55 ± 0.08	96.73
Saline-Treated	7	21.0 ± 1.4	7.78 ± 0.13	3.67 ± 0.03	-

[a] $\frac{\text{Liver Weight}}{\text{Body Weight}}$, expressed as percent of saline-treated controls.

[b] Mean ± Standard Error.

TABLE 6. Comparison of Microsomal Values for Xylose- and Saline-Treated Rats

	Days of Administration	Microsomal Protein Concentration[a]	Cytochrome P-450[b]	Ethylmorphine Demethylase[c]	Aniline Hydroxylase[c]
Xylose-Treated	7	24.90 ± 0.36[d]	0.447 ± 0.019	6.76 ± 0.27	0.457 ± 0.015
Saline-Treated	7	24.08 ± 0.27	0.468 ± 0.017	6.97 ± 0.27	0.433 ± 0.017

[a]Expressed as mg protein per gram liver.

[b]Expressed as nmol of cytochrome P-450 per mg microsomal protein.

[c]Expressed as nmol product formed per mg microsomal protein per minute.

[d]Mean ± Standard Error.

control animals showed no evidence of fatty infiltration. This fatty infiltration was characterized by spherical accumulation of fat droplets in the hepatocyte cytoplasm. Using an osmium tetroxide technique, these fat droplets were impregnated with a black stain, which more clearly identified them as being fatty infiltration. These fatty changes occurred primarily in the portal areas, although mild, diffuse, hepatocellular infiltration was noted throughout the lobules.

The glucose and fructose groups appeared to have a 40% reduction in liver glycogen content, compared to the saline-treated control group, using histologic techniques. Quantitative assays confirmed that the glycogen content was significantly decreased ($p < 0.05$) in both the glucose- and fructose-treated groups after seven days of treatment, being 67% and 72% of control, respectively (Table 7).

Antipyrine Half-Life Study

During the seven days of injections, weight gain and animal condition were similar to that of the hepatic microsome study. The *in vivo* assessment of glucose and fructose on antipyrine pharmacokinetics demonstrated that each hexose sugar prolonged the mean blood half-life of antipyrine 73% and 72%, respectively, and decreased the metabolic clearance rate to 53% and 51% of control, respectively (Table 8). The mean correlation coefficient of linear elimination of ^{14}C-antipyrine was 0.992 (range: 0.998 to 0.974). The half-life was calculated from the concentrations of the unchanged drug in whole blood from 30 to 240 minutes after ^{14}C-antipyrine administration. The mean antipyrine half-life for the saline-treated control animals (86.3 minutes [range: 69.5 to 104.0 minutes]) was significantly less ($p < 0.05$) than that of either the glucose- or fructose-treated animals

TABLE 7. Comparison of Liver Glycogen after Seven Days of Treatment with Glucose, Fructose or Saline

Groups	Liver Glycogen mg/gm	Liver Glycogen Percent of Saline-Treated Controls
Glucose-Treated	35.8 ± 3.0[a,b]	67.3[b]
Fructose-Treated	38.4 ± 2.1[b]	72.2[b]
Saline-Treated	53.2 ± 3.8	-

[a]Mean ± Standard Error.

[b]Significantly different from corresponding saline-treated controls, $p < 0.05$.

TABLE 8. *In Vivo* Assessment of Glucose and Fructose on Antipyrine Pharmacokinetics

	Body Weight (gm)	Volume of Distribution (ml)	Half-Life (min)	Metabolic Clearance Rate (MCR) (ml/min)
Glucose-Treated	202 ± 5[a]	134 ± 5	149.7 ± 9.8[b]	0.633 ± 0.048[b]
Fructose-Treated	207 ± 6	132 ± 7	148.8 ± 8.0[b]	0.613 ± 0.34[b]
Saline-Treated	211 ± 5	149 ± 5	86.3 ± 6.4	1.196 ± 0.039

[a]Mean ± Standard Error.

[b]Significantly different from corresponding saline-treated controls, $p < 0.05$.

(149.7 minutes [127.5 to 169.3 minutes] and 148.8 minutes [127.0 to 172.6 minutes], respectively). The volume of distribution was not significantly different between groups, being 149 ml (range: 140 to 167 ml), 134 ml (120 to 147 ml), and 132 ml (117 to 158 ml) for the saline-, glucose-, and fructose-treated groups, respectively. The metabolic clearance rate for the saline control group was 1.20 ml/min (range: 1.08 to 1.30 ml/min), significantly greater ($p < 0.05$) than the metabolic clearance rates for the glucose- and fructose-treated groups, which were 0.633 ml/min (0.489 to 0.772 ml/min) and 0.613 ml/min (0.467 to 0.702 ml/min), respectively.

Serum Hexose Study

Serum assays conducted 48 hours after the start of glucose administration (Table 9) revealed that the glucose-treated groups maintained significantly elevated ($p < 0.05$) serum glucose levels. The serum glucose levels at 1, 2, and 3 hours after glucose injection were 193 ± 7, 165 ± 2, and 147 ± 6 mg/dl, respectively. Serum fructose levels in the glucose-treated group were too low to measure by the techniques we used (<5 mg/dl).

The fructose-treated groups maintained significant elevations ($p < 0.05$) in both serum glucose and serum fructose levels. Serum glucose levels at 1, 2, and 3 hours after treatment were 149 ± 10, 140 ± 7, and 151 ± 7 mg/dl. Although these levels were significantly higher than control values, they were significantly lower ($p < 0.05$) than the glucose-treated rats at both one hour (149 ± 10 vs. 193 ± 7 mg/dl) and two hours (140 ± 7 vs. 165 ± 2 mg/dl). At three hours

TABLE 9. Comparison of Serum Glucose, Fructose and Protein Levels for Experimental and Control Rats

	Time (hours > 16th injection)	Glucose (mg/dl)	Fructose (mg/dl)	Total Protein (gm/dl)
Glucose-Treated	1	193 ± 7[a,b]	<5	7.0 ± 0.3
	2	165 ± 2[b]	<5	6.6 ± 0.2
	3	147 ± 6[b]	<5	6.8 ± 0.4
Fructose-Treated	1	149 ± 10[b]	47 ± 4[b]	6.8 ± 0.2
	2	140 ± 7[b]	34 ± 2[b]	7.1 ± 0.4
	3	151 ± 7[b]	24 ± 2[b]	6.6 ± 0.1
Saline-Treated	1	89 ± 2	<5	6.7 ± 0.2

[a]Mean ± Standard Error.

[b]Significantly different from corresponding saline-treated controls, $p < 0.05$.

serum glucose levels were similar between the two hexose groups. Serum fructose levels were 47 ± 4, 34 ± 2, and 24 ± 2 mg/dl for hours 1, 2, and 3, respectively.

The serum glucose levels in the saline-treated control groups were 89 ± 2 mg/dl at one-hour post-injection. These levels were not significantly different from the fasting (12 hours) serum glucose levels of six untreated rats of the same age and weight, which were maintained on the standard rat diet. The serum glucose levels in these animals were 85 ± 8 mg/dl. The serum fructose levels in the saline-treated controls were less than the detectable level of the test (<5 mg/dl).

Total serum protein levels were not significantly different between the carbohydrate-treated and the saline-treated groups (Table 9). In addition, protein levels did not significantly vary within the carbohydrate groups following the injections.

COMMENTS

The parenteral administration of glucose and fructose to rats generally decreased hepatic MFO activity, but the pattern of change exhibited considerable temporal variation. The administration of either carbohydrate for two days resulted in a small, but nonsignificant decrease of both cytochrome P-450 content and ethylmorphine activity; these decreases were significantly lower than normal control values at five and seven days. Although aniline hydroxylase activity was not significantly decreased after two or five days of glucose or fructose administration, at seven days it was significantly lower than that of control rats. This earlier response of ethylmorphine to carbohydrate administration may be due to the fact that cytochrome P-450 exists in multiple forms (30,67). Thus, the specific form involved in ethylmorphine N-demethylation may be more susceptible to carbohydrate administration than the form associated with aniline hydroxylation.

The *in vitro* techniques used in these measurements may have considerably affected the data. In the intact hepatocyte, the endoplasmic reticulum is a network of lipoprotein tubules. These tubules extend throughout the cytoplasm and are continuous with both cellular and nuclear membranes (49). However, in making *in vitro* measurements, the microsomes are fragmented and form small vesicles. Enzymes in this fragmented form may act much differently from the way these same enzymes function in the intact membrane (64). Therefore, antipyrine half-life studies were done as an *in vivo* assessment of such potential differences. Antipyrine is negligibly bound to plasma proteins (9), and its decay in plasma results, almost entirely, from its metabolism

in the liver (71,76,77). Antipyrine measurements supported those made *in vitro* on hepatic MFO activities.

There were differences in the osmolarity of the glucose, fructose, and saline injections. However, there did not appear to be any resultant effects due to the hyperosmolar carbohydrate solutions compared to the iso-osmolar solution. Also, there were no apparent differences in the state of hydration between any of the groups, since the serum protein levels and the volumes of distribution were similar.

Decreased hepatic MFO activity clearly resulted, in some manner, from carbohydrate administration. However, the exact way in which these carbohydrates reduced MFO activities is not established. It is known that nutritional imbalances can affect microsomal enzyme activity (12,19,39,40,53), but this was probably not the case in this study, since all of the rats received diets that had identical amounts of calories, protein, and carbohydrate. Although the experimental and control animals received identical amounts of these dietary constituents, it is recognized that the observed fatty infiltration in the liver could have resulted from overall nutritional inadequacies. It is known that high dietary glucose levels can increase the liver's capacity to convert glucose and acetate into fatty acids, as well as cause significant elevations in α-glycerophosphate dehydrogenase (23,24,33). The latter enzyme is responsible for the production of the glycerol moiety of neutral fats. Lipogenesis could also have been stimulated by glucose oxidation in the direct (hexose monophosphate) oxidative pathway, by the production of $NADP^+$ (6,22). Since fatty acids undergo

oxidative metabolism by the hepatic MFO system (52), the decreased hepatic MFO activity and the increased lipogenesis could have accounted for the lipid accumulation in the liver (29).

Several possible mechanisms will now be discussed for the decreased cytochrome P-450 content, and the subsequent losses of MFO activity, seen in the present study. Among these are hyperglycemia, hypoinsulinemia, and increased levels of hepatic cyclic AMP. We observed hyperglycemia, in the range of 140 to 193 mg/dl, in both glucose- and fructose-treated rats. Our observations confirm those of others (63,72) who described similar reductions in hepatic microsomal drug metabolism when high carbohydrate diets were fed. Even though these investigators failed to detect significant elevations in blood glucose, hyperglycemia might have occurred since glycosuria was consistently observed in overnight urine collections from their rats (63,72). In another study (1) on the effects of hyperglycemia on drug metabolism, a 24-hour infusion of glucose failed to produce any effect on the _in vitro_ metabolism of hexobarbital. This is not surprising, however, since results from the present study show that _in vitro_ drug metabolism is not significantly decreased until five days of sustained hyperglycemia.

It was interesting that administration of either glucose or fructose resulted in a constant state of hyperglycemia. Studies by Fitch _et al_. (23,24) showed that the four enzymes necessary for conversion of fructose to glucose (aldolase, fructokinase, fructose 1,6-diphosphatase, and glucose-6-phosphatase) can be induced in rats fed a high-fructose diet. Therefore, in the present study, the

fructose-induced hyperglycemia may have resulted from a stimulation of these enzymes, producing the gluconeogenic effect (48).

A second possibility for the decreased hepatic MFO activity is hypoinsulinemia. A recent report by Ackerman and Leibman (1) showed that depressed insulin levels in rats decrease hepatic MFO activity. Since glucose- and fructose-treated rats in the present study may have become functionally insulin-depleted during attempted adjustments to the newly-imposed hyperglycemia, functional hypoinsulinemia may account for the decreased MFO activity that we observed. The possibility also exists that the high levels of parenterally-administered glucose may have caused mild degenerative changes in the pancreatic beta cells. Degenerative changes in the beta cells have been reported in cats receiving intraperitoneal glucose injections (20). These degenerative lesions could also result in hypoinsulinemia.

A third explanation for decreased MFO activity is that increased levels of hepatic cyclic AMP may have resulted from hypoinsulinemia, or from some other unidentified, initiating factor. Hypoinsulinemia concurrently produces increased hepatic cyclic AMP levels (1,62) and decreased hepatic MFO activities (1,78,80). Recent evidence indicates that this inhibition of hepatic microsomal drug metabolism by cyclic AMP is mediated by production of an inhibitory substance in the microsomes (79). Cyclic AMP elevation is suggested in this study by decreased hepatic glycogen levels. Both the glucose- and fructose-treated groups showed a significant decrease in hepatic glycogen content after seven days of treatment. Elevation of cyclic AMP could

result in increased phosphorylase _a_ activity and increased glycogenolysis, while at the same time, reducing glycogen synthesis (59).

The decreased hepatic MFO activity seen in this study, as a result of parenteral carbohydrate administration, could result in decreased metabolism of exogenous compounds and endogenous steroids in the body. This decrease in drug metabolism could, in turn, permit the accumulation of exogenous chemicals or drugs in the body, with potentially detrimental effects.

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